REMARKS

Reconsideration of the above-identified patent application in view of the proposed amendment above and the remarks below is respectfully requested.

No claims are herein proposed to be canceled or added in this paper. Claims 1, 26, 27 and 30 are herein proposed to be amended in this paper. Therefore, claims 1-9, 11-16, 18-24, 26-27 and 30 are pending and are under active consideration.

Claims 1-6, 8, 11, 18-25, 28 and 29 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Rice *et al.* (Oncogene (1998) 17, 1807-1812) in view of Gifford (US 5750335)." In support of the rejection, the Patent Office states the following:

> Rice *et al.* teach a method for identifying 5-methylcytosine positions in a sample genomic DNA, said method comprising the steps of:
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> (a) chemically treating a sample genomic DNA obtained from at least one cell in such a way that cytosine and 5-methylcytosine react differently and from products with different base pairing behavior (p. 1811, treatment with sodium bisulfite);
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> (b) amplifying by means of a polymerase reaction a segment of the genomic DNA obtained in step (a) (p. 1811, second column);
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> (c) performing steps (a) and (b) on a reference genomic DNA (Figure 3, methylation was determined for eight different cell types);
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> Rice *et al.* utilize chemical treatment with sodium bisulfite in a method to identify the location of methylated cytosines in genomic DNA. Treatment with bisulfite results in the conversion of unmethylated cytosine residues to uracil, while methylated cytosine residues remain unchanged. Thus, in a sample with unmethylated sequence (for example the HMEC) there would be no change in sequence, but in a sample with high levels of methylation, after PCR there would be thymines where the methylatd cytosines previously were located. Rice *et al.* effectively introduce mutations nucleic acid sequences via the treatment with sodium bisulfite. Rice et al. utilize a sequencing method to determine the methylation positions after amplification of the sequences.
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> ...

Rice et al. do not form heteroduplexes from the amplified products for the comparison of a test and reference sample.

Gifford teaches a method for identifying sequence differences between two nucleic acids that comprises the steps of:

(d) forming heteroduplexes from two different nucleic acid samples (Col. 3, lines 40-50);

(e) introducing a detectable label into the heteroduplexes of step (d) by means of a reaction which is specific for non-complementary base pairs (Col. 4, lines 15-20), and

(f) determining the position of 5-methylcytosine in the sample genomic DNA based on the presence and position of the detectable label (Col. 4, lines 5-10, 20-25).

Gifford specifically teaches comparing a sample (patient) nucleic acid fragment with a control (normal) nucleic acid fragment (Figure 2).

...

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methylation detection method taught by Rice *et al.* so as to have utilized the mutation detection methods taught by Gifford. One would have been motivated to utilize the methods taught by Gifford in order to achieve the express benefits of the methods taught by Gifford which include achieving "rapid and accurate genetic screening and diagnosis by comparing two nucleic acids for differences in their sequences...to locate previously unknown mutations of a nucleotide sequence, and to identify the sequence itself, where the nature and position of the mutation within a region of the genome is unknown, and where the location of the region itself is unknown (Col. 3, lines 25-40)." With regard to claim 6, the result required in claim 6 would have been a necessary property of the practice of the assay taught by Rice *et al.* in view of Gifford. Namely, where there was differential methylation between the sample genomic DNA and any of the reference DNA's taught by Rice *et al.*, when these were subjected to heteroduplex analysis as taught by Gifford erroneous base pairings would have been produced at the positions at which 5-methylcytosine was located in the sample genomic DNA but not in the reference genomic DNA.

Later in the Office Action, the Patent Office states the following:

With regard to the reiterated 103 rejections, which are modified to address the newly added and amended claims, applicant first discusses the benefits of the instant invention at pages 18-19. Applicant points out that Rice et al. rely on sequencing the sample in order to determine a methylation pattern. It is noted that this is a piecemeal analysis of only Rice et al., which does not address the totality of the rejection. The rejection specifically discusses the modification of the methods taught by Rice et al. so as to use the heteroduplex analysis as taught by Gifford for the determination of methylation patterns. Nonetheless, it is further noted that the instant claims do not exclude the use of a sequencing step, for example, after fragments containing heteroduplexes are identified, as the claims are broadly drawn using "comprising" language.

Applicant argues that nothing in Gifford teaches or suggests step (f) in claim 1. Gifford does teach identifying the position of mismatched nucleotides, as is cited in the office action. The statement that Gifford specifically teaches detecting methylation is a typographical error. Clearly, the examiner pointed to the portion of Gifford where Gifford teaches identifying the position of a mismatch between two sequences. Rice et al. provides a method for modifying genomic DNA at positions of methylation (i.e. introducing mismatches using bisulfite). Thus, as stated in the rejection, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used the methods taught by Herman to identify mismatches among the sequences being inquired by Hall et al[1]. Thus, the identification of these mismatches using the method taught by Gifford would have necessarily resulted in the determination of the position of the 5-methylcytosines in the genomic DNA, as the mismatched sequences among the different cell lines would represent portions of the genome that are differentially methylated from one genomic DNA sample to another. Applicant further argues that it would have required hindsight reconstruction to arrive at the instant invention. However, as discussed in the rejection, Gifford teaches a number of different reasons why one would have been motivated to have utilized the methods taught therein for the detection of sequence differences between two sequences. Rice et al. undertake a method to introduce sequence differences where methylation is differential between two sequences.

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[1] Applicants query what is meant by the references to Herman and Hall et al. in this sentence since these references are not included in the statement of the rejection as being relied upon by the Patent Office. Clarification is respectfully requested.

> Thus, in view of the teachings of the benefits of the methodology taught by Gifford, one would have been motivated to utilize heteroduplex analysis as taught by Gifford in order to have attained the benefits taught by Gifford. In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon highsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In this case, the benefits of using the method taught by Gifford are clearly taught by Griffin. Thus, as stated in the rejection, it would have been obvious to have used the methods taught by Gifford to detect the mismatches between sequences introduced by Rice et al. into the sample and reference genomic DNA molecules. The rejections are MAINTAINED and applied to the newly added and amended claims.

Insofar as the subject rejection pertains to claims 25, 28 and 29, Applicant respectfully submits that the rejection is in error since claims 25, 28 and 29 were canceled in a previous amendment. Insofar as the subject rejection pertains to claims 1-6, 8, 11 and 18-24, Applicant respectfully traverses the subject rejection.

At the outset, Applicant notes that claim 1, from which claims 2-6, 8 and 18-24 depend, and claim 30, from which claim 11 depends, have been amended herein to replace the transition term "comprising" with the transition term "consisting of." In view of this amendment, the claims at issue now specifically exclude a sequencing step after fragments containing heteroduplexes are identified. Therefore, to the extent that the Patent Office's conclusion of obviousness is predicated on the claims being broad enough to include a sequencing step after the identification of heteroduplexes, Applicant respectfully submits that such a rationale is no longer applicable.

Moreover, for at least the reasons below, Applicant respectfully submits that there is no basis for combining the teachings of the references in the manner proposed by the Patent Office.

Rice et al., which is relied upon by the Patent Office for allegedly teaching above steps (a)-(c), is directed at a study in which BRCA1 promoter segments from various breast cancer cells and normal cells were fully sequenced, and a comparison was thereafter made as to the cytosine methylation sites of the different segments. Because methylated and unmethylated cytosine bases cannot be distinguished using typical sequencing protocols, the Rice promoter segments were treated, prior to sequencing, with bisulfite in such a manner that the unmethylated cytosine bases were converted to uracil whereas the methylated cytosine bases were unchanged.

Rice et al. does not teach or suggest any of steps (d)-(f) of claims 1 or 30. Consequently, the Patent Office proposes combining Rice et al. with Gifford. Gifford, however, is directed at a method for screening for genetic variation, the method involving hybridizing a "test" sequence, i.e., a potential variant nucleic acid sequence from a patient with a nucleic acid standard sequence. If the test and standard (reference) nucleic acids contain one or more nucleotide sequence differences, then the double stranded nucleic acid formed from hybridization of the sequences will contain one or more nucleotide pair mismatches, i.e., the hybrid will comprise a heteroduplex. Detection of the presence of a heteroduplex is effected through the use of mismatch binding proteins. It should be noted that none of the hybridized sequences of Gifford are bisulfite-treated sequences.

The Patent Office is apparently taking the position that it would have been obvious to use the Gifford heteroduplex technique to identify nucleotide pair mismatches present in hybrids formed between normal and cancerous Rice segments that had previously been treated with bisulfite and then amplified. One problem with this rationale, however, is that it presupposes that one of ordinary

skill in the art at the time of the invention would have been interested in knowing the sites of cytosine methylation in a sample segment, without also knowing the entire sequence of the segment. In the absence of such an interest, there would have been no reason to form the proposed hybrids. Applicant respectfully submits that the references, themselves, provide no teaching or suggestion that such information would be desirable. Instead, Rice et al. seems to suggest just the opposite since it involves sequencing the entire segment. Moreover, Gifford does not cure this deficiency since, as noted previously, Gifford does not provide any teaching or suggestion relating to cytosine methylation.

Furthermore, Applicant wishes to point out to the Patent Office the following aspects of bisulfite treatment that are apparently not being given weight by the Patent Office: First, bisulfite treatment results in conversion of unmethylated cytosine to uracil. **Methylated cytosine, by contrast, is unaffected by bisulfite treatment**. As can readily be appreciated, given the typically high ratio of unmethylated cytosine to methylated cytosine in a given nucleic acid sequence, bisulfite treatment typically results in a dramatic change to the treated sequence. Consequently, to the extent that the Patent Office's comments appear to envision bisulfite treatment being used as a mechanism for introducing a mutation at a point of methylation, the Patent Office is in error on multiple accounts. Second, bisulfite treatment cannot be carried out on double stranded DNA, but rather, only on single stranded DNA. Therefore, the DNA must first be denatured; subsequent to the treatment procedure, the two strands do not normally anneal due to the differences in sequence between sense and antisense strands. Accordingly, mismatching cannot occur. The Patent Office states that it would have been obvious to identify these mismatches with the Gifford method. However, it is not clear how this could be effected since, contrary to the Patent Office's suggestion,

the Rice method does not result in the formation of mismatches at positions of methylated cytosines. Third, the bisulfite conversion of methylated DNA is not equivalent to the introduction of a point mutation. In the art, a mutation is considered to be a random event (methodologies for introducing such mutations including exposure to carcinogens and/or X-rays). The specific and deliberate conversion of one base to another cannot thus be considered to be equivalent to artificially introducing mutations.

The mere fact that the teachings of the references **may** be combined or modified does not necessarily render the invention obvious; instead, there must be some teaching or suggestion in the prior art of the desirability of the combination or modification. See In re Gordon, 221 USPQ 1125 (Fed. Cir. 1984). As noted by the Patent Office, Gifford teaches that its method provides "rapid and accurate genetic screening and diagnosis by comparing two nucleic acids for differences in their sequences...to locate previously unknown mutations of a nucleotide sequence, and to identify the sequence itself, where the nature and position of the mutation within a region of the genome is unknown and where the location of the region itself is unknown." However, the foregoing statement is merely a statement of the advantages of the Gifford method for **mutation analysis**. Such a statement provides no motivation for a person of ordinary skill in the art to apply this approach to the analysis of DNA **methylation**, which is not a mutation, but rather, is a problem of a different nature that does not lend itself to analysis using mutation analysis techniques. Thus, Gifford is directed at identifying mutations, which is a different problem than that confronted in Rice and in the present invention, namely, that of identifying differentially methylated positions.

Moreover, Rice not only enables one to identify differentially methylated cytosines but also is expressly suited to the ascertainment of the sequence context in which those methylated positions

lie. Gifford does not enable one to ascertain the sequence of the genomic region of interest. Therefore, a person of ordinary skill in the art would not have been motivated to modify the teachings of Rice with those of Gifford to arrive at the method of claim 1 as to do so would result in the loss of the benefit of ascertaining the sequence context in which the differentially methylated positions occur.

Accordingly, for at least the above reasons, the present rejection should be withdrawn.

Claims 9, 11-17, 26-27 and 30 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Rice *et al.* in view of Gifford as applied to claims 1-5, 7-8, 11, 18-25, 28, and 29 above, and further in view of Koster *et al.* (US 6428955)." In support of the rejection, the Patent Office states the following:

> The teachings of Rice *et al.* in view of Gifford are applied herein as applied in the previous rejection. In the method taught by Rice *et al.* in view of Gifford, sodium bisulfite is used which results in the modification of unmethylated cytosines, and therefore mismatches would occur at positions where cytosine was located in the genomic DNA. Rice *et al.* in view of Gifford do not teach a method in which the heteroduplex is detected by cleavage of the heteroduplex molecule or in which mass spectrometry is used to analyze the size of the DNA fragments.
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> With regard to claims 11-14, Koster *et al.* teach methods for analyzing the size of nucleic acid fragments using mass spectrometry, specifically teaching the use of MALDI-TOF and ESI (Col. 18, line 66-Col. 19, line 11).
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> With regard to claim 15, which requires that the nucleic acids in step (e) are "adapted" to the performance capacity of the mass spectrometer, Koster *et al.* teach utilizing a variety of PCR amplification methods to obtain PCR products that they analyze using the mass spec (See examples 14-15, for example). With regard to claim 16, Koster *et al.* teach utilizing nested PCR to amplify products for detection (Examples 5 and 14, for example), a method which uses primers that are set stepwise along the DNA with respect to the inner and outer pairs of primers and produce a series of amplification

products, at least one of which is within the mass range detectable by means of mass spectrometry. These PCR primers are considered to be "set stepwise" since they amplify two differently sized products where one is a size "step" down from the other.

Claim 26 differs from claim 1 in that in step (b) a primer used in the PCR is fluorescently labeled and provided with a chemical function thereby enabling the immobilization of the ampliicate on the surface, step (e) utilizes a chemical mismatch cleavage methodology, and step (f) utilizes mass spectrometry, wherein in step (g) the presence or presence and position of the 5-methylcytosine within the genomic DNA is deduced from the length of the cleaved nucleic acids. Claim 27 is similar to claim 26 but requires that a detectable label is introduced into the heteroduplex by an enzymatic reaction which is specific for non-complementary base pairs. This limitation is provided in the methods taught by Rice *et al.* in view of Gifford.

Likewise, newly added claim 30 is similar to claim 26, and thus also similar to claim 1. Steps (a)-(d) of claim 30 are similar to those of claim 1. Unlike claim 1, however, claim 30 requires that the heteroduplexes of step (d) are cleaved by a chemical mismatch cleavage reaction. Claim 9 depends from claim 30 and requires that the nucleic acid backbone of the heteroduplex is specifically cleaved at the non-complementarily base paired positions by an enzymatic means.

Koster *et al.* teaches a method in which a heteroduplex is cleaved by an agent that cleaves the unhybridized portion so that a mismatch results in two products and then detecting these by mass spectrometry to detect the presence of the mismatch (Col. 5, lines 30-40; Col. 23, lines 25-40). Koster *et al.* further teach primers that are labeled with biotin (a means for immobilizing an amplificate on a surface; col. 35, for example) and primers that are labeled with a radioactive label and oligonucleotides that are fluorescently labeled (Col. 49, for example). In addition, as noted previously in this office action, any nucleic acid sequence itself is considered a "chemical function" that would enable the immobilization of the amplificate on a surface. Claims 26 and 27 never actually require the immobilization of the amplificate on a surface, only that such immobilization is "enabled." Nonetheless, Koster further teach methods in which the sequence to be detected is immobilized to a solid support by means of hybridization (Col. 3, lines 60-67).

> It would have been prima facie obvious to one of ordinary skill in the art to have modified the method taught by Rice *et al.* in view of Gifford *et al.* so as to have used the amplification and detection methods taught by Koster *et al.* One would have been motivated to use mass spectrometry as a means for detection of nucleic acid fragments in order to take advantage of the express benefits of such a method as taught by Koster *et al.*, who state "the processes of the invention provide for increased accuracy and reliability of nucleic acid detection by mass spectrometry (Col. 5, lines 62-65). Furthermore, it would have been prima facie obvious to have utilized fluorescently labeled primers in place of the radioactively labeled primers taught by Koster *et al.* in order to have provided an alternative labeling method that is safer to use as opposed to using radioactivity in the laboratory.

Insofar as the present rejection pertains to claim 17, Applicant respectfully submits that the rejection is in error since claim 17 was canceled in a previous amendment. Insofar as the present rejection pertains to claims 9, 11-16, 26-27 and 30, Applicant respectfully traverses the rejection.

Claims 9 and 11-16 depend from claim 30. Claim 30 is patentable over Rice et al. in view of Gifford for at least the same types of reasons discussed in the previous rejection. Kolster fails to cure the deficiencies of Rice et al. and Gifford. Therefore, claims 9 and 11-16 are patentable over the combination of Rice et al., Gifford and Kolster.

Claims 26 and 27 are patentable over the applied combination of references for at least the same types of reasons given above for claim 1 and/or claim 30.

Accordingly, for at least the above reasons, the present rejection should be withdrawn.

Claim 7 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Rice *et al.* in view of Gifford as applied to claims 1-5, 7-8, 11, 18-25, 28, and 29 above, and further in view of Nazarenko *et al.* (US 6090552)." In support of the rejection, the Patent Office states the following:

> The teachings of Rice et al. in view of Gifford are applied herein as applied in the previous rejection. Rice et al. in view of

> Gifford do not teach methods wherein the reference DNA is methylated at all CpG positions.
>
> However, the inclusion of a methylated control in an assay for the determination of methylation would have been routine at the time the invention was made. Nazarenko et al. teach methods for detecting methylation in samples, and teach the inclusion of methylated control nucleic acids in these assays and in kits for performing these assays (see Col. 37-38 and 49-50, for example). Thus, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have included in the assays taught by Rice et al. in view of Nazarenko et al. a control which is methylated at all CpG positions in order to have had a standard for comparison of all results against those obtained with a methylated control.

Applicant respectfully traverses the rejection. Claim 7 depends from claim 1. Claim 1 is patentable over Rice et al. in view of Gifford for at least the same types of reasons discussed above. Nazarenko et al. fails to cure the deficiencies of Rice et al. and Gifford. Therefore, claim 7 is patentable over the combination of Rice et al., Gifford and Nazarenko et al.

Accordingly, for at least the above reasons, the present rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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Dated: April 11, 2005

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on April 11, 2005.

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